REMARKS

THE AMENDMENTS

Applicants cancel claims 46-51, 55, 56, and 60, without prejudice. Applicants also amend claims 45, 52, 53, and 57, and add new claims 61-76. Applicants reserve the right to prosecute cancelled claims and other claims based on their subject matter in this or other applications. Cancellation of claims and claim amendments are to expedite prosecution of the application. Claim amendments and new claims add no new subject matter, and are fully supported throughout the specification and by the drawings and claims as filed. Support and reasoning for the amendments are provided below.

Support for Claim Amendments and Reasons for Amendments

These amendments are made to clarify the claims and to expedite allowance of the present application.

Claim 45 has been amended so that it is more particularly drawn to the use of extracts from *Drosophila* embryos. The claim has also been amended to remove the requirement that the results of the method results in a greater amount of protein produced from a capped and polyadenylated template than from a non-capped or non-polyadenylated template.

Support for the method can be found in the specification on page 3, lines 17-23:

> According to a second aspect of the invention there is provided a method for the preparation of a *Drosophila* embryo extract comprising the steps of a) dechorionating *Drosophila* embryos in an aqueous isotonic buffer comprising detergent and bleach; b) washing the embryos; c) homogenizing the embryos to produce a homogenate; d) centrifuging the homogenate; and e) recovering non-pelleted material from the centrifuged homogenate.

and on page 5, lines 25-27:

> According to a third aspect of the invention there is provided the extract of *Drosophila* embryos or ovaries, or of mammalian cells, produced according to the methods of the second aspect of the present invention.

Finally on page 5, line 28 extending to page 6, line 3:

> According to a fourth aspect of the invention there is provided a method for the *in vitro* translation of a ribonucleic acid template, comprising the steps of adding a ribonucleic acid template to a translation mix in the presence of the embryo, ovary or mammalian cell extract of the third aspect of the invention to form a reaction mix and incubating the reaction mix for at least 90 minutes at between 18°C and 28°C.

Support for the method can also be found in Examples 1, 2, and 3, in which *Drosophila* embryo extracts are prepared (Example 1), the translation conditions are optimized (Example 2), and the results of the translations are compared with results using an alternative method (Example 3).

Claims 46-51 are canceled without prejudice.

Claims 52 and 53 that further specify steps of the method of extract preparation were previously filed and have been amended only to change their dependency, such that they are now dependent on amended claim 45.

Claim 54 was previously presented.

Claims 55 and 56 are canceled without prejudice.

Claim 57 is drawn to the components of the reaction mix recited in Claim 45. Claim 57 has been amended such that the claim recites "said translation mix comprises" rather than "said conditions comprise" to conform to the language of newly amended Claim 45. In addition, "a reducing agent" has been removed from the list of components of the reaction mix. The presence of $Mg(OAc)_2$ in the reaction mix is provided in new Claim 62. The presence of KOAc in the reaction mix is provided in new Claim 63. The presence of DTT in the reaction mix is provided in new Claim 64. Amended Claim 57 and new Claims 62, 63, and 64 reflect the translation conditions laid forth in the specification. For example, in referring to the composition of the reaction mix, the specification states on page 6 (lines 5-9):

> By reaction mix is meant a solution of components necessary for *in vitro* translation to occur. Such components may include any of the following: spermidine, amino acids, creatine phosphate, creatine kinase, dithiothreitol (DTT), buffer, $Mg(OAc)_2$, KOAc, tRNA, cell extract and RNA tempate.

The specification further indicates on page 6, lines 14-15: "The concentrations of DTT, $Mg(OAc)_2$, and KOAc, should be optimized for each individual mRNA template, as will be appreciated by those skiled in the art." On page 7, lines 1-2, the specification states: "However, final concentration of $Mg(OAc)_2$ can be in the range 0-3 mM. KOAc can range between 0 and 200 mM. DTT can range between 0-4 mM." As laid out in the specification, amended Claim 45 and new claims 62-64 include the both the absence and presence of $Mg(OAc)_2$, KOAc, and DTT in the reaction mix.

Claim 58 has been amended to make the language conform to that of amended Claim 45 ("wherein said incubating is" has replaced "wherein said conditions comprise"). Claim 58 has also been amended to change the temperature of incubation from "between about 18 degrees Celsius to about 37 degrees Celsius" to "between about 18 degrees Celsius and about 28 degrees Celsius". Amended Claim 45, from which Claim 58 depends, is now drawn to the use of *Drosophila* embryo extract rather than an animal cell extract. In referring to the *Drosophila* extract, the specification on page 6, lines 2-3 refers to "incubating the reaction mix for at least 90 minutes at between 18°C and 28°C."

Amended Claim 59 has also been changed to conform to the language used in amended Claim 45 ("wherein said incubating is" has replaced "wherein said conditions comprise"). Support for amended Claim 59 can be found in the specification on page 6, lines 3-4: "Preferably, the reaction mix is incubated for at least 90 minutes at 25°C."

Claim 60 is canceled without prejudice.

Claim 61 has been previously presented.

New claims 62-64 are discussed above, in the context amended claim 57, which also is directed toward the composition of the translation mix.

New claim 65 recites a method for producing a protein by *in vitro* translation using a Hela cell extract made by hypotonic lysis and homogenization of harvested Hela cells. Support for new Claim 65 can be found on page 5, lines 3-6:

> The invention also provides a method for the preparation of a mammalian cell extract comprising the steps of a) collecting cells by centrifugation; b) washing the cells; c) resuspending the cells; d) homogenizing the cells to produce a homogenate; e) centrifuging the homogenate and f) recovering non-pelleted material from the centrifuged homogenate. This cell extract is well-suited to the use in the method of the first aspect of the invention.

Lines 11-24 on page 5 further describe the steps of preparing the mammalian cell extract. Example 4 describes the use of the method in producing a Hela cell extract to be used in translation reactions, and translation reactions that employ Hela cell extracts are described in Example 5.

Support for new Claim 66, drawn to the components of the hypotonic buffer recited in claim 65, can be found on page 5, lines 13-17:

> For preparation of extract, the cells should be washed with phosphate buffered saline at 4°C, before resuspension in a suitable ice cold hypotonic buffer such as a buffer containing containing Hepes 10 mM. PH 7.6 KOAc 10mM. $Mg(OAc)_2$ 0.5 mM and Dithiothreitol 5 mM.

Support for new claim 67, specifying centrifugation force, can be found in the specification on page 5 in line 23: "The homogenate is then centrifuged, ideally at 14000g for 5 minutes at 4°C." It is also supported in Example 4: "Cell lysates were centrifuged at 14,000 g for 5 minutes (a longer centrifugation is not recommended) at 4°C." (page 20, lines 1-2).

Support for new Claim 68, regarding the absence of nuclease treatment, can also be found on page 5, lines 3-6 of the specification, where the preparation of a mammalian cell extracts is disclosed:

> The invention also provides a method for the preparation of a mammalian cell extract comprising the steps of a) collecting cells by centrifugation; b) washing the cells; c) resuspending the cells; d) homogenizing the cells to produce a homogenate;

> e) centrifuging the homogenate and f) recovering non-pelleted material from the centrifuged homogenate. This cell extract is well-suited to the use in the method of the first aspect of the invention.

Lines 11-24 on page 5 further describe in detail the method of preparing a mammalian cell extract, in which a nuclease treatment is not included. Example 4 describes specifically the method in producing a Hela cell extract to be used in translation reactions, where the method lacks a nuclease step. In Example 5, in which the Hela cell extract is used in a translation reaction, the extract is referred to as : "untreated extract" (page 20, line 6).

Support for new Claims 69, 70, and 71 can be found on page 6, lines 5-8:

> By reaction mix is meant a solution of components necessary for *in vitro* translation to occur. Such components may include any of the following: spermidine, amino acids, creatine phosphate, creatine kinase, dithiothreitol (DTT), buffer, $Mg(OAc)_2$, KOAc, tRNA, cell extract and RNA template.

In Example 5, on page 20, lines 5-19, the listed reagents for a reaction mix using a Hela cell extract also include ATP (line 12) and GTP (line 13). In this specific example, DTT is not present in the reaction mix.

New claim 73, reciting an incubation temperature of about 37 degrees Celsius finds support on page 20 of the specification, lines 18-19: "Reaction mixtures, typically at a final volume of 12.5 µl were incubated at 37°C for 90 minutes."

New claims 74, 75, and 76, reciting reaction times of at least 25 minutes, at least 45 minutes, and at least 90 minutes, find support in Figures 13A and 13B. These figures present data on the translation of luciferase transcripts as detailed in Example 5. It can be seen from these graphs that at 25 minutes, 45 minutes, and 60 minutes, the amount of capped and polyadenylated transcript that was translated (white bars) exceeded the amount amounts of transcript that was capped only (bars with diagonals) or polyadenylated only (vertically striped bars) added together. Thus the method exhibits the

synergistic effects of translating RNAs having both a poly A tail and a cap structure at all of these temperatures.

OBJECTIONS TO FIGURES

Figure 11

Applicants acknowledge the receipt and acceptance by the Examiner of corrected Figure 11.

Figure 12

Applicants note that the Examiner has objected to Figures 12 and 13 as presenting contradictory results. This objection was asserted in the Office Action mailed August 7, 2003 for Figures 12A and B. Applicants believe that in the recent Office Action mailed March 2, 2004, the Examiner intended to state that Figures 12A and 12B were objected to rather than Figures 12 and 13, as Figures 12A and 12B, and not Figure 13, are discussed by the Examiner in both the recent and previous Office Actions. Applicants will therefore regard the objection as pertaining to Figures 12A and 12B.

Applicants are not aware of drawings being objected to for contradictory results and respectfully request that the Examiner provide guidance as to the section of the MPEP that refers to such objection in order that we can respond appropriately.

Applicants do however welcome the opportunity to more fully explain Figures 12A and 12B, and Applicants' assertion that these figures do not present contradictory results. In doing so, we present 1) the experiments that produced the results provided graphically in Figures 12A and 12B, 2) the results presented in Figures 12A and 12B, and 3) the significance of the results.

RNA Stability Experiment of Figure 12

The present invention provides an *in vitro* translation system that recapitulates the increased efficiency of *in vivo* translation of messages that are both polyadenylated and capped. The inventors demonstrate (for example, in Figures 1 and 2) that use of their translation system results in a large synergistic increase in translation of messages that are both polyadenylated and capped when compared with messages that are polyadenylated but not capped, or capped but not polyadenylated.

As a supplementary study, the inventors sought to determine to what degree (if any) the stability of the capped and/or polyadenylated messages in the translation mixes contributed to the enhancement of translation.

The experiments that resulted in the data shown in Figure 12 were translation experiments performed as described in Example 5. In these experiments, as disclosed on page 20, line 25 through page 21, line 3:

> Two sets of transcripts coding for the reporter enzymes CAT (Preiss T. and Hentze M. W. 1998 Nature 392: 516-520) and firefly luciferase. Luc (Iizuka N. et al. 1994 Mole. Cell. Biol. 14: 7322-7330) were tested in the translation assay and the functional half life of each transcript has been determined as follows:
>
> - 5' capped (Cap, m7Gppp) — 40 minutes
> - 5' capped and polyadenylated (pA, 98 adenines) — 55 minutes
> - uncapped — <10 minutes
> - uncapped and polyadenylated — <10 minutes

The information in this paragraph, which introduces the discussion of Figure 12, can be rephrased as follows: Four *RNA transcripts* were tested for their *stability* in the translation system. Thus: These figures do *not* show results of time optimization of the translation reaction – no protein products are monitored. We see only *transcript levels at various timepoints in translation reactions*, measured by use of a phosphoimager on a Northern blot. Surveying the list in the above passage, we see that the four transcripts can be summarized as 1) + cap, -poly A ; 2) + cap, +poly A ; 3) – cap, - poly A; and 4) –cap; + poly A. The data provided with the list of the four transcripts is the half-life of each radiolabeled transcript in minutes.

Results of Experiment Depicted in Figure 12

From these data it is clear that having a cap structure (1 and 2) greatly increases the stability of the message. Uncapped messages (3 and 4) have much shorter half-lives by comparison. The data also show that polyadenylation does not contribute much to RNA stability. Transcript 4, which is polyadenylated, has about the same half-life as transcript 3, which is not.

The following paragraph of the specification, page 21, lines 4-7, refers specifically to the data of Figure 12:

Figure 12 shows a northern blot analysis of the transcripts during the translation assay. Bars represent radioactive intensity of each messenger measured by a phosphoimager. Graph (A) shows the four messengers at different times, from 0 to 150 min. Graph (B) shows the Cap and the Cap-pA transcripts from 0 to 90 min.

Comparing the phosphoimager intensity units on the y-axis of each graph indicates that the samples used in Figure 12A and 12B were from different experiments, as at time zero there was a much greater incorporation of label into the RNA transcripts in the experiment associated with Figure 12B than there was into the RNA transcripts in the experiment associated with Figure 12A.

At time zero, each transcript starts with a certain level of signal based on the efficiency of incorporation of radiolabel into that transcript. Here, the experiments do not compare levels of different transcripts with one another, but rather, *to what degree the incorporated label of each message in the assay declines over time.*

The discrepancy between the relative amounts of incorporated label of Cap and Cap-pA message in Figure 12A and Figure 12B at the zero time point indicate that these two graphs provide data from different experimental samples. At the outset of the experiment of Figure 12A, there was more label incorporation into the "Cap only" message than into the "Cap-pA" message, whereas at the outset of the experiment of Figure 12B, there was more label incorporation into the "Cap-pA" message than into the "Cap only" message. Within each experiment/figure, it is the comparison of the amount of each transcript at the incubation time points with its starting amount that is relevant in these experiments.

In Figure 12A, when one surveys the time points sample by sample, it is clear that while the two messages that lack a Cap structure (horizontally striped bars and blank bars) decline precipitously from their starting levels throughout the assay until they cannot be detected at 150 minutes, the two messages having a Cap structure (diagonally striped bars and vertically striped bars) are remarkably stable through at least 90 minutes of translation assay.

In Figure 12B, the levels of just capped messages are shown. Again, comparing radioactivity levels at the progressive time points with the amounts at

the zero time point, the graph shows that both of these messages are stable for at least 60 minutes, after which time the capped message lacking poly A declines.

Significance of Results of Experiment Depicted in Figure 12

The data shown in Figures 12A and 12B demonstrates RNA stability only. The data do not report on translational efficiency. The data indicate that the cap structure enhances the stability of RNA transcripts in translation reactions, whereas a poly A tail contributes little to RNA transcript stability. Therefore the synergistic effect of the cap structure and poly A tail on the efficiency of translation of RNA transcripts in translation systems of the present invention cannot be attributed to their combined effect on the stability of the transcripts, since the presence of a poly A tail does not contribute to RNA stability in these translation system.

This result, while an important scientific finding, does not bear on the patentability of the invention. The synergistic enhancement of translation of transcripts that are both capped and polyadenylated in the system developed by the inventors (as depicted in Figure 13) remains a key feature of the invention, whether due in any part to transcript stability or not.

Declaration

The Examiner has objected to the declaration because of non-initialed and non-dated alterations. Applicants respectfully request that this matter be held in abeyance until allowable subject matter has been indicated.

CLAIMS ARE ENABLED UNDER 35USC §112, FIRST PARAGRAPH

The Examiner alleges that claims 45-60 are not enabled under 35 USC §112, First Paragraph because claim 45 is not enabled for methods of translation other than those that use *Drosophila* embryo extracts and incubation times of at least 90 minutes. Applicants disagree that the specification is not enabling for translation methods other than those using *Drosophila* embryo extracts and incubation times of at least 90 minutes. However, to expedite allowance of claims, applicants have cancelled some claims, amended some claims, and presented new claims.

Applicants have amended independent claim 45 such that it is now directed toward the use of a *Drosophila* embryo extract, rather than an animal cell extract, in translation reactions. Amended claims 52, 53, 57, 58, and 59; previously presented claims 54 and 61, and new claims 62-64 are dependent on amended independent claim 45. Thus, claims 45, 52-54, 57-59, and 61-64 all have as subject matter methods of *in vitro* translation using a *Drosophila* embryo extract. Amended claim 45 recites a *Drosophila* embryo extract made by a method comprising dechorionating *Drosophila* embryos in an aqueous isotonic solution comprising detergent and bleach. The specification provides enabling disclosure of dechorionation of *Drosophila* embryos beginning on page 3, line 16, and extending to page 4, line 21, and also in Example 1. In Example 2, the specification provides disclosure on performing translation reactions using *Drosophila* embryo extract and optimization of concentrations of various reaction components. In Example 3, translation reactions using *Drosophila* embryo extract made using the methods of the present invention and *Drosophila* embryo extract made using the methods of Scott et al. (1979) are compared. The data from these experiments are shown in figures 1 and 2. In these figures, it can clearly be seen that translation efficiency of capped and polyadenylated messages is synergistic (with respect to messages that are capped only or polyadenylated only) at both of the time points surveyed (45 minutes and 90 minutes). Therefore, applicants contend that the present invention is enabled for times less than 90 minutes. Applicants further assert that, given that the synergistic effect was seen at 45 minutes and at 90 minutes when all other conditions were constant as taught in the specification (for example, reaction temperature and reagent concentrations), testing of

translation efficiencies for reactions performed additional time periods of less than 90 minutes would not constitute undue experimentation.

Applicants have also submitted new claim independent claim 65, which is drawn to *in vitro* translation using a Hela cell extract. Enabling disclosure of the production of the Hela cell extract, as recited in part a) of the claim, is found in Example 4 the specification, pages 19-20. The use of the Hela cell extract in translation assays is provided in Example 5, pages 20-21. Data resulting from these assays is depicted in Figure 13. The Examiner asserts that Example 5 does not provide support for the synergy effect, stating "As can be seen by comparing Fig. 12A and 12B, the amounts of translation products for Cap-pA and Cap mRNAs after 60 minutes and 90 minutes are not in the same proportion: in Fig. 12A, the amount of Cap-pA is lower than the amount of Cap mRNA, while the reverse is shown for the same time periods in Fig. 12B . . ." Applicants disagree, as Figure 12 does not show amounts of translation products. Rather, Figures 12A and 12B depict results of two experiments on the stability of RNA transcripts through the course of translation reactions. Applicants have provided a full explanation of the experiment depicted in Figure 12 on pages 10-13 of this response.

Figure 13 does provide data on the efficiency of *in vitro* translation of capped and polyadenylated RNA transcripts using a Hela cell extract made by the methods of the present invention. In Figure 13A, translation product resulting from capped and polyadenylated transcript (ClucA; white bars) at 25, 45, and 90 minutes far exceed the additive amount of translation product resulting from capped transcript (Cluc, diagonally striped bars) and polyadenylated transcript (lucA; vertically striped bars). The same data is depicted in Figure 13B on a graph with a reduced scale on the y-axis. Thus, the specification does provide evidence that a Hela cell extract prepared by resuspending Hela cells in a hypotonic buffer, homogenizing the cells, centrifuging the homogenate, and removing the supernatant results can translate capped and polyadenylated transcripts with much greater efficiency than transcripts that are capped only or polyadenylated only.

CLAIMS ARE DEFINITE UNDER 35USC §112, SECOND PARAGRAPH

The Examiner alleges that claims 45-60 are indefinite under 35 USC §112, Second Paragraph. The Examiner finds claim 45 indefinite in part b) in which the encoded protein produced is greater than the total of i) the amount of encoded protein produced from a message having a 5' cap but not a 3' poly A tail, plus ii) the amount of encoded protein produced from a message having a 3' poly A tail but not a 5' cap.

Applicants have amended claim 45 and removed this wording from claim 45. Applicants therefore respectfully request that the rejection be removed.

The Examiner also alleges claim 45 is indefinite for failing to specify whether the term "ribonucleic acid template" refers to endogenous mRNA, exogenous mRNA, or both. Applicants have amended claim 45 to change the term "ribonucleic acid template" to "exogenous ribonucleic acid template". Applicants therefore respectfully request that the rejection be removed.

CLAIMS ARE NOVEL UNDER 35 USC §102

The Examiner has rejected claims 45-47 and 57-60 under 35 USC §102(b) as allegedly anticipated by Iizuka et al. (Mol. Cell Bio., 14: 7322-7330, 1994). Applicants have amended claim 45 such that the claim now recites a Drosophila embryo extract prepared by a method comprising dechorionating Drosophila embryos in an aqueous isotonic buffer comprising detergent and bleach. Iizuka et al. do not teach or suggest a Drosophila embryo extract. Claims 52-54, 57-59, and 61-64 depend from amended claim 45. Claim 45 and claims 52-54, 57-59, and 61-64 are therefore novel under 35 USC §102(b). Applicants therefore respectfully request that the rejection be removed.

New claim 65 recites a method for producing a protein by in vitro translation using a Hela cell extract produced by harvesting Hela cells grown in culture, centrifuging the Hela cells, resuspending the Hela cells in a hypotonic buffer, lysing the Hela cells with a homogenizer, centrifuging the Hela cell homogenate for 5 minutes or less, and removing the supernatant of the centrifuged lysate. Iizuka et al. do not disclose a Hela cell extract made in this manner. New claims 66-75 depend from new claim 65. New claim 65 and new claims 66-75 therefore are novel under 35 USC §102(b), and Applicants therefore respectfully request that the rejection be removed.

CLAIMS ARE NONOBVIOUS UNDER 35 USC §103

The Examiner has rejected claims 48-50 under 35 USC §103(a) as allegedly unpatentable over Iizuka et al. (Mol. Cell Bio., 14: 7322-7330, 1994) in view of Scott et al. (Biochemistry, 18: 1588-1594, 1979). Applicants have canceled claims 48-40, reserving the right to prosecute those claims in this or future applications. Applicants therefore respectfully request that the rejection be removed.

Applicants submit that the claims are ready for examination and in condition for allowance.

Respectfully submitted,

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In the event this paper is deemed not timely filed the applicants hereby petition for an appropriate extension of time. The fee for this extension may be charged to Deposit Account No. 501,321 along with any other additional fees which may be required with respect to this paper; any overpayment should be credited to the account. If any fees charged to this account will exceed $500, applicants respectfully requests that its counsel be notified of such amounts before the Deposit Account is charged.